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Set-up of a system to reliably measure the startle response in marmoset monkeys; application in animal models of anxiety and psychosis

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Hierbij zenden wij u het TNO-rapport PML 1998-B42 getiteld 'Set-up of a system to reliably measure the startle response in marmoset monkeys; application in animal models of anxiety and psychosis', geschreven door Dr. B.P.C. Melchers, B. Groen, R.A.P. Vanwersch, Drs. I.H.C.H.M. Philippens en Dr. P.L.B. Bruijnzeel. Deze studie is gefinancierd vanuit de basissubsidie in combinatie met een bijdrage van Solvay Duphar.

In dit rapport worden experimenten beschreven om in de marmosetaap een model te ontwikkelen voor pathologische angst alsmede een model voor psychose. Bij beide onderwerpen wordt gebruikgemaakt van een reflex, de zogenaamde startle response. Bij beide ziektebeelden zijn er verstoringen zichtbaar in deze reflex. Er is een aantal in de mens werkzame farmaca getest om te onderzoeken in hoeverre de modellen aan de verwachtingen voldoen. Hoewel, mede door het tekort aan apen, er geen statistisch significante verschillen zijn aangetoond, lijkt de trend te zijn dat de farmaca inderdaad hun bedoelde werking hebben.

Mocht u vragen hebben betreffende de inhoud van het rapport of de uitvoering van het onderzoek dan kunt u altijd contact opnemen met de heer dr. B.P.C. Melchers. Het doorkiesnummer is aan de bovenzijde van deze brief vermeld.

Ir. M. van Zelm,
Directeur Programma.

Managementuittreksel

Titel	:	Set-up of a system to reliably measure the startle response in marmoset monkeys; application in animal models of anxiety and psychosis
Auteur(s)	:	Dr. B.P.C. Melchers, B. Groen, R.A.P. Vanwersch, Drs. I.H.C.H.M. Philippens en Dr. P.L.B. Bruijnzeel
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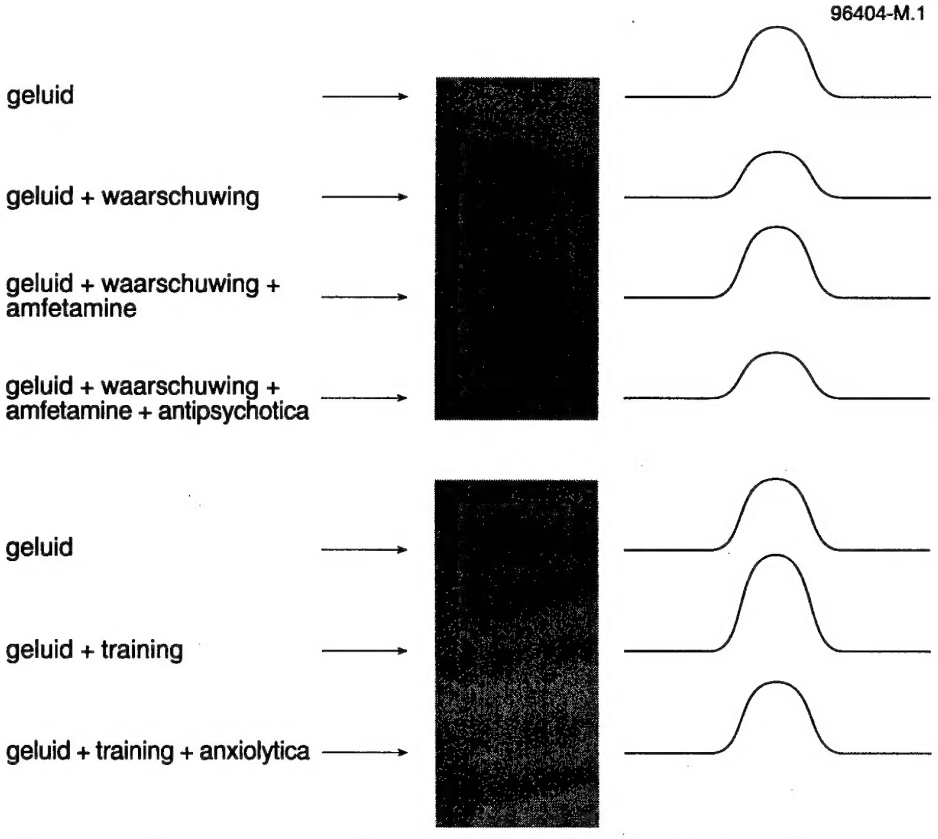
In dit rapport worden experimenten beschreven met het doel een methode op te zetten om de acoustische startle reflex (ASR) in marmosetapen te kunnen meten. De ASR is een reflexmatige motorische reactie ten gevolge van een (harde) geluidsstimulus. Met behulp van deze ASR is het onder andere mogelijk een tweetal psychiatrische ziektebeelden in een proefdier na te bootsen.
Patiënten die leiden aan schizofrenie hebben een verstoring in de zogenaamde prepulse inhibitie (PPI) van de ASR. Onder normale omstandigheden is de ASR sterk verminderd als de startle stimulus wordt voorafgegaan door een 'waarschuwingssignaal' (de term waarschuwingssignaal staat hier tussen aanhalingstekens omdat er bij de PPI van de ASR geen sprake is van een bewust proces). Deze PPI treedt bij schizofreniepatiënten niet of slechts in geringe mate op. In proefdieren is het mogelijk de PPI te verminderen door het geven van hallucinogene stoffen. Geneesmiddelen met een antipsychotische werking kunnen veelal de PPI weer normaliseren.
Pathologische angst: in proefdieren kan een vorm van geconditioneerde angst worden opgewekt door een specifieke situatie (bijvoorbeeld licht uit) steeds gepaard te laten gaan met een aversieve stimulus. Wanneer tijdens deze specifieke situatie een startle stimulus wordt gegeven zal de ASR groter zijn dan normaal, er is een zogenaamde fear-potentiation (FPS) van de ASR opgetreden. Deze fear-potentiation kan, in principe, worden tegengegaan door het geven van anxiolytica.
Uit het rapport blijkt dat we er in zijn geslaagd een techniek te ontwikkelen waarmee de ASR op betrouwbare wijze in marmosetapen gemeten kan worden. Met behulp van deze opstelling is aangetoond dat er in de marmosetaap, net als in andere diersoorten inclusief de mens, een duidelijke PPI wordt gevonden. Vervolgens is het duidelijk dat in de marmoset een duidelijke fear-potentiatie van de ASR kan worden opgewekt. Concluderend kan worden gesteld dat we hiermee een model in handen hebben waarmee potentieel de antipsychotische of anxiolytische werking van stoffen kan worden onderzocht.
In een serie vervolgexperimenten is getracht dit model te valideren. Hiertoe zijn de effecten van een aantal stoffen met een bekende anxiolytische c.q. psychotische werking in dit model getest. De effecten van deze stoffen zijn vergeleken met de effecten van 'negatieve controles', dat wil zeggen stoffen waarvan bekend is dat ze

geen effect hebben in de onderhavige modelsystemen. Sommige van de geteste farmaca hadden een duidelijk effect op de startle signalen, echter er bleken geen significante effecten te zijn op de fear-potentiatie of op de PPI. Dit laatste door een vrij grote spreiding in de meetresultaten. Er was echter wel duidelijk sprake van een trend in die zin dat bijvoorbeeld Diazepam en in mindere mate Fluvoxamine, een effect op de fear-potentiatie leken te hebben. Buspiron leek een bifasische dose-response curve te hebben; Haloperidol had geen effect op de FPS. Iets soortgelijks gold voor de PPI: Ketamine en Quinpirol leken een effect te hebben op de PPI, Diazepam niet. Amfetamine is waarschijnlijk in een te lage dosering gegeven om effect te kunnen hebben. Concluderend lijken beide modellen zeker potentie te hebben, echter, er zullen waarschijnlijk meer dieren per behandelingsgroep moeten worden gebruikt en/of er zal een methode gevonden moeten worden om de spreiding te verminderen. Dit laatste is misschien mogelijk door een voorselectie van dieren met de hoogste FPS te maken. Een mogelijk alternatief kan zijn om de procedure om de dieren te trainen verder aan te scherpen. Misschien is het dan mogelijk om de FPS in de verschillende dieren meer gelijk te trekken. Dit zou mogelijk kunnen gebeuren door bepaalde dieren meer trainings-trials te geven of door bepaalde dieren 'herinnerings'-trials tijdens de test toe te dienen.



Figuur M.1: Visualisatie van de Startle-responsen bij verschillende behandelingen.

Contents

1 Introduction

The auditory or acoustic startle reflex is a motor response following an intense sound stimulus. This reflex has a very short latency (Ison et al., 1973), indicating that a very simple neuronal circuit, comprising only a few synapses, is involved. Indeed, lesion and stimulation studies (see Davis et al., 1982 and the references therein) have shown that the primary startle circuit maximally consists of only six synapses: I) from the auditory nerve to the ventral cochlear nucleus; II) to the nuclei of the lateral lemniscus; III) to the nucleus reticularis pontis caudalis; IV) to the spinal interneuron; V) to the lower motor neurone; VI) to the muscle. However, the amplitude of the acoustic startle reflex appears to be under the control of higher brain centres, for instance, a state of (conditioned) anxiety potentiates the startle reflex. This effect appears to be under the control of the amygdala (Davis, 1992). A conditioned fear potentiation of the startle response is seen when the startle reflex is elicited in the presence of a cue (versus the absence of this cue) previously paired with a shock. Fear potentiation of the startle is now commonly used as a sensitive test for the anxiolytic potency of drugs (e.g. Davis, 1979, 1990; Hijzen et al., 1995). Fear potentiation of the startle response, as measured by the eyeblink reflex, can also be elicited in humans (Grillon et al., 1991).
One of the features of the startle reflex is that it shows pre-pulse inhibition. When a sound stimulus of low intensity is applied briefly, i.e. within a period of about 50-100 msec, before the startle stimulus, the amplitude of the startle reflex is decreased (e.g. Hoffman and Ison, 1980). This feature of the acoustic startle reflex is being used in animal models for disturbed sensory gating. It has been shown that patients suffering from schizophrenia demonstrate an impaired pre-pulse inhibition of the startle reflex (Grillon et al., 1992). In experimental animals, a similar situation can be induced by giving the animals hallucinogenic drugs like apomorphine (Swerdlow et al., 1994) or phencyclidine (Wiley, 1994).

1.1 Neurotransmitter systems involved in anxiety

The classic hypothesis infers 5-HT as the major neurotransmitter involved in anxiety. A reduction of the activity of 5-HT pathways appears to have an anxiolytic effect. An activation of these pathways is anxiogenic (for review see Griebel et al., 1995 and the references therein). Several studies (see Griebel et al., 1995) have shown that this hypothesis is at least partly true, although the picture is undoubtedly more complicated and probably many more neurotransmitter systems, like the GABAergic system, are involved.

1.2 Neurotransmitter systems involved in psychosis/schizophrenia

One of the main hypotheses of the underlying cause of the symptoms of schizophrenia is a hyperactivity of the dopaminergic system. Neuroleptics, the main drugs used as a therapy against schizophrenia, act as dopamine antagonists and overactivation of dopaminergic neurones (e.g. by amphetamine, a drug that leads to an increased release of dopamine) may lead to symptoms of psychosis. In addition, one of the possible side-effects of anti-Parkinson drugs, which are directed to stimulate the dopaminergic system, is a drug-induced psychosis. However, there are many reports on post-mortem tissue of schizophrenics showing that various transmitter systems may have been affected: e.g. the nicotinic cholinergic system (Freedman et al., 1995), the GABAergic system (Simpson et al., 1989) and the glutaminergic system (Sherman et al., 1991; Harrison et al., 1991, Halberstadt, 1995).

1.3 Why a primate?

The main argument is found in the fact that primates are our closest animal relatives. Therefore, intuitively, it appears that the chance that a monkey will react in a similar way to drugs as we do is much greater than when a rodent is used. However, this intuitive argument receives support from neuro-anatomical studies showing, for example, that there is much more similarity of the regional distribution through the hippocampus of several neurotransmitter receptor types, including the 5-HT_1 receptor, of marmosets and humans than of rats and humans (Kraemer et al., 1995). Furthermore, dopaminergic projections to the hippocampus are much more dense in primates than in rodents (Samson et al., 1990), the distribution over the brain of D1 and D2 dopaminergic receptors may differ between rodents and primates (Meador-Woodruff et al., 1991; Camps et al., 1990) and, in contrast with rodents, a high concentration of cholecystokinine-A receptors, presumably associated with dopaminergic cells, is found in primates (Hill et al., 1990). In addition, there appears to be a difference in the D4 receptor gene between primates (including humans and marmosets) and rats (Matsumoto et al., 1995).

In this study, we establish a system which allows us to measure the startle response in marmoset monkeys reliably. In addition, we have explored whether it is possible to use these systems in the search for the effectiveness of anti-psychotics and anti-anxiety agents.

2 Materials and methods

2.1 Animals

A total of 21 common marmosets (Callithrix jacchus), obtained from the breeding colony of the Biomedical Primate Research Centre in Rijswijk, The Netherlands, was used for this study. The animals weighed between 220g and 400g, were individually housed and given water and food ad libitum. Most animals did not receive any drugs however. Four animals were only used to see whether the dark-light protocol in the startle set-up induced extra fear and were not tested in any other way. Three animals were used both for the initial experiments and for the final validation experiment. These animals were used in the pre-testing period for PPI and in the validation sessions for FPS tests.

2.2 Startle apparatus

The final version of the startle apparatus consists of a plexiglass cylinder, closed at the top with a very fine wire mesh. The bottom of the cylinder consists of metal rods that deliver the aversive stimulus to the animals. The aversive stimulus consists of a current of 300-500 μA delivered by a constant current source controlled by an IBM compatible personal computer (PC). The current is scrambled, using a custom built scrambling device, over the 14 rods as square current pulses of 7 msec duration. Sound stimuli (20 ms, 70-120 dBA) are generated by the same PC, amplified using an Akai AM-17 amplifier and applied to the animals by a tweeter (piezo KSN 1086A, Telec, the Netherlands). The tweeter is placed 12 cm above the top of the plexiglas cylinder. The plexiglass cylinder is placed upon a platform connected to 3 pressure transducers. This whole system is placed in a sound attenuated box of 42x67x70 cm. The box may be illuminated with a 40W light bulb. The output signal of the pressure transducers is amplified and filtered using custom built amplifiers/filters and fed into the analogue digital converter in the PC (I/O card, PCL-812PG, Advantech Co Ltd). Startle signals recorded by these pressure transducers are averaged and stored on disk for later analysis. The strength of the sound stimuli is checked regularly by means of an Eagle Db 120 (Telec, the Netherlands) sound strength measuring device.

2.3 General procedure

The animal was put into the plexiglass cylinder and placed in the startle set-up. Thereafter, the experiment was started. The number of startle stimuli that was administered to the animal depended on the type of experiment conducted. The

computer was programmed in such a way that sound stimuli could be delivered, in random order, with different strengths and/or at different intervals. In addition, the PC controlled the illumination (i.e. on or off) of the sound attenuated box. The startle stimuli were delivered in a semi-random way, i.e. at an interval of 20 ± 4 seconds. To prevent large variations in the startle signals due to movements of the animals unrelated to the startle stimulus, the startle stimuli were given only when the animal did not move. This was determined by ascertaining whether the output signal of the pressure transducers was within 50 units of the baseline for a period of at least 100 msec.

2.4 Drugs

Ketamine was obtained from Tesink veterinary productions; Haloperidol, Quinpirol, Buspiron, and Amphetamine were purchased from Research Biochemicals International. Diazepam was purchased from Roche. Fluvoxamine was a kind gift from Dr. J. van der Heyden, Solvay Duphar (Weesp, The Netherlands).

2.5 Dosage

For the fear potentiated startle, Diazepam and Buspiron were given at a dose of 0.3 (low), 1 (middle) and 3 (high) mg/kg; Haloperidol at a dose of 0.03, 0.1 and 0.3 whereas Fluvoxamine 1, 3 and 10 mg/kg.
For the pre-pulse inhibition experiments, Diazepam was given in the same dose as above. Ketamine was also given in the same dose. Amphetamine was given in a dose of 0.1, 0.3 and 1 mg /kg and Quinpirol in a dose of 0.03, 0.1 and 0.3 mg/kg.

3 Results

3.1 Test development

First, some pilot experiments were performed to develop a suitable startle set-up for marmosets based on our experience in rodents (Philippens et al., 1997). In the latter system, the startle response of the hind legs of the animals is recorded, whereas in most commercially available systems, a whole body response is usually measured. Recording of the movement of the hind legs only leads to relatively easy interpretable startle responses (see Figure 1). In our initial experiments, it appeared to be impossible to immobilize a marmoset monkey in a similar way as a rat or guinea pig. The animals always managed to struggle themselves into such a position that their hind legs were not in contact with the plateau to which the force transducers were connected. For this reason, we were forced to return to the rather simple system shown in Figure 2. In this system, the monkey could move freely in the plexiglass chamber and a whole body startle reflex was recorded. Using this set-up, a characteristic startle signal could be obtained with first a positive peak, followed by a negative peak, followed again by a positive peak (Figure 3). We quantified the startle response either by taking the overall area under the curve (AUC), the values of the maximal positive or negative peaks or the differential value of the two latter peaks. There was a high correlation between the AUC and the differential peak amplitude of the startle reflex (r=0.98 for 36 startle signals).

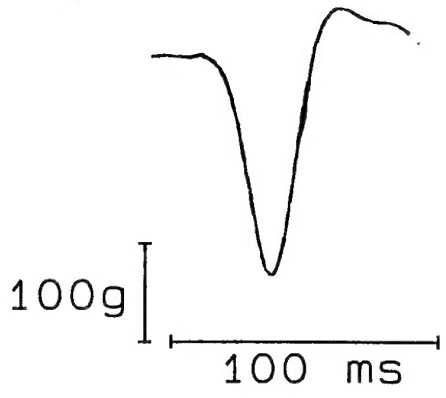


Figure 1: Mean auditory startle induced by a 120 dBA startle stimulus in a guinea pig (mean of 6 animals).

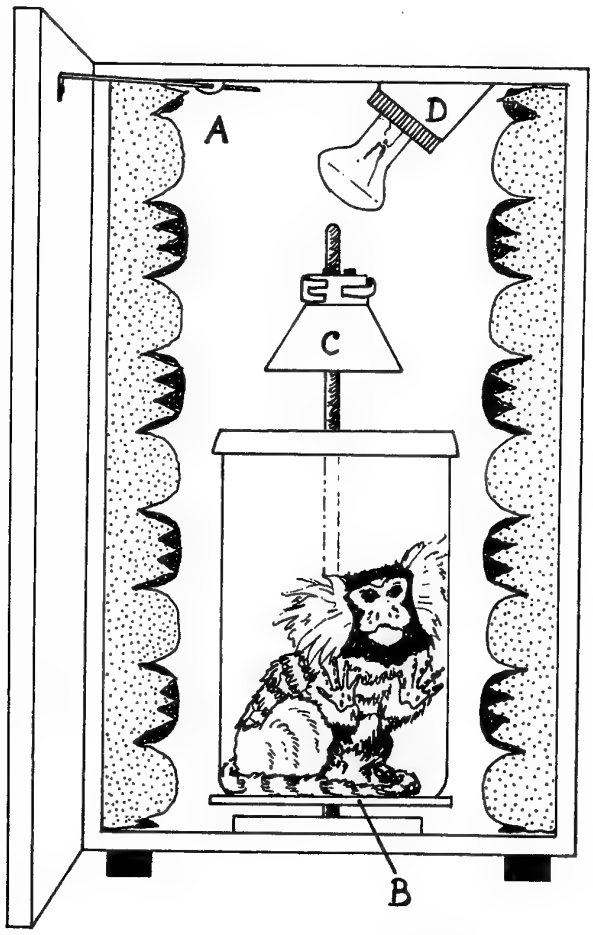


Figure 2: *Startle system for marmosets. A: sound attenuated chamber. B: the platform to which the pressure transducer recording the signal is connected. C: loud-speaker, used for application of both prepulse and startle sound stimuli. D: light-bulb for the illumination of the chamber. For the fear conditioning, the light was turned off.*

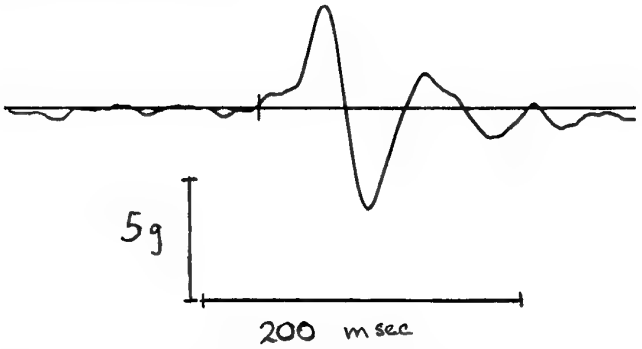


Figure 3: *Representative auditory startle response recorded in a marmoset monkey.*

3.1.1 Effect of startle stimulus intensity on startle amplitude

At first a set of experiments was performed to investigate the effect of the intensity of the startle stimulus on the amplitude of the startle response. Four different intensities were tested: 70, 80, 100 and 120 dBA. The results are shown in Figure 4. At 70 BA, only a very small startle response could be measured (0.92 ± 0.12 g), which was, however, still clearly detectable above the background. Under similar conditions to when the startle responses were recorded, but without startle stimuli, a background with a peak-peak value of 0.4 ± 0.06 g (n=4 trials of 1 marmoset) was found. At 120 dBA, the startle responses usually reach about 4 g (peak-peak). However, this value may vary enormously between individual animals. On the basis of these data, it was decided to use 120 dBA as the startle stimulus and to use a pre-pulse intensity of 70 dBA in the following experiments.

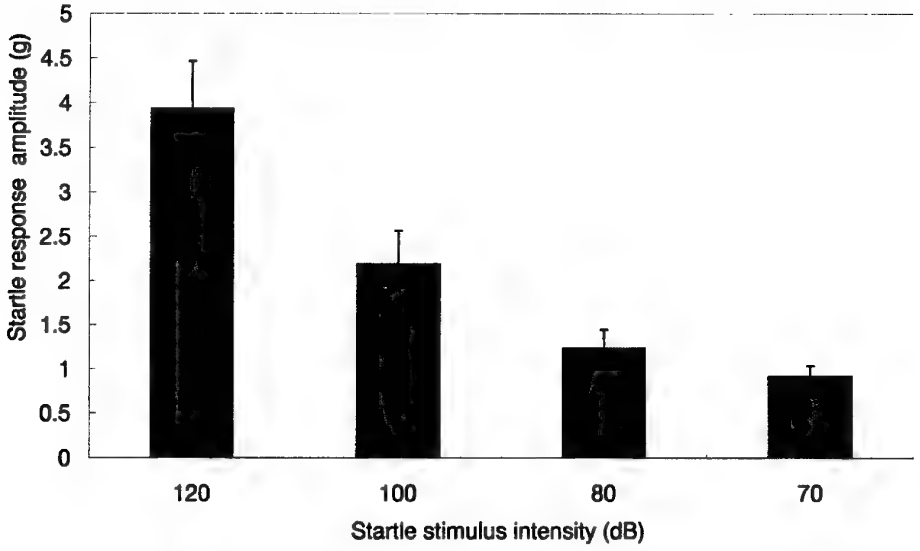


Figure 4: Effect of the strength of the startle stimulus on the startle amplitude (differential peak amplitude). The mean ± s.e.m. of the responses of 4 animals is shown.

3.1.2 Effect of pre-pulse-stimulus interval on startle amplitude

As expected, marmosets showed a clear pre-pulse inhibition of the startle response. There appeared to be little effect of the interval between the pre-pulse and the startle stimulus (tested range 50-150 msec). At all three intervals, a pre-pulse inhibition of about 50% was found (Figure 5).

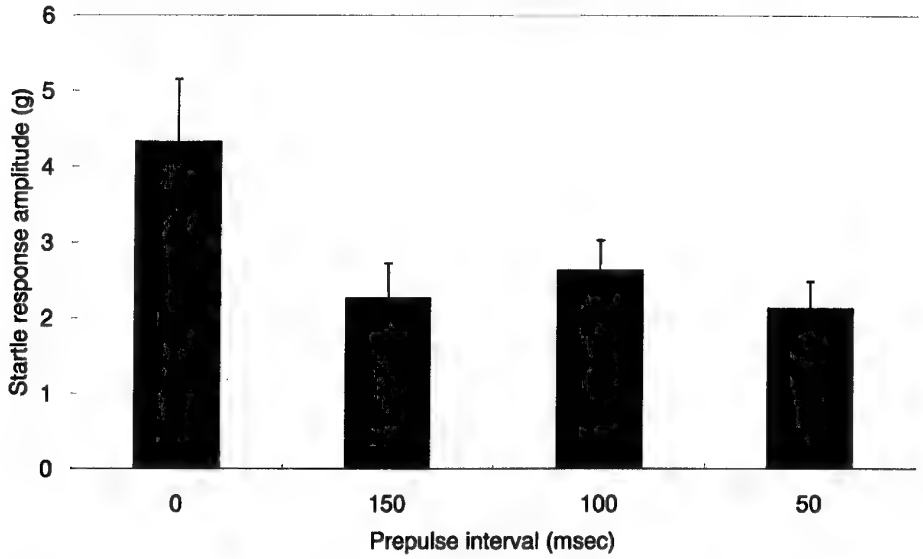


Figure 5: *Pre-pulse inhibition of the startle response (differential peak amplitude) at different intervals between the pre-pulse and the startle stimulus. The mean ± s.e.m. of the responses of 4 animals is shown.*

3.1.3 Fear potentiation of the startle response

First, the pain threshold of the animals was determined, to be able to establish the current amplitude to be used as aversive stimulus. This was done by increasing the current on the gridfloor of the startle chamber while registering the movements of the animals. It appeared that most animals started to move around at 350 μA. Therefore, the strength of the aversive stimulus was set initially at this value. The protocol used was essentially the same as used in rats (Hijzen et al., 1995): on day one, 15 conditioning trials were given with an interval of about 20 seconds. Each trial consisted of a period of 4 seconds in which the light in the startle chamber was turned down; during the last 0.5 second of the dark period, the aversive stimulus was applied, i.e. a current of 350 μA on the gridfloor. On the next day the startle responses in light and dark were recorded. It appeared that in all animals the startle response was larger in the dark than in the light period (a mean value of 141 ± 17%, n=4 animals), i.e. apparently a fear potentiation had occurred. However, using a similar dark-light protocol in 4 naïve animals (i.e. not conditioned), a similar 'fear potentiation' was found; in 3 out of 4 animals the startle responses were larger in the dark than in the light period (mean value: 136 ± 23%).
Because the fear potentiation was not as large as we hoped for, we proceeded by changing the protocol in the following way: 2 animals of the 4 used in the first fear potentiation experiment and 2 naïve animals were trained for 2 consecutive days, 15 trials a day. In addition, the aversive stimulus was increased up to 500 μA. Before doing this, the two 'new' animals had also been subjected to the same training protocol (using an aversive stimulus of 350 μA). The change of the protocol seemed to have the desired effect: on the third day, when the fear potentiation of the startle was determined, a fear potentiation of 188% was found. This same

procedure was repeated the next week, with roughly the same result. Thereafter the animals had a resting period of about 1.5 weeks. The animals were not conditioned in this period but they were tested in the dark-light protocol, again with a similar result. A renewed conditioning period of two consecutive days with 15 trials per day also resulted in a fear potentiation of about 180%. These results are compiled in Figure 6.

In 3 out of 4 animals, no appreciable effects of the fear potentiation protocol were found on the startle response amplitude in the light period; however, in one animal the control startle response increased tremendously (Figure 7).

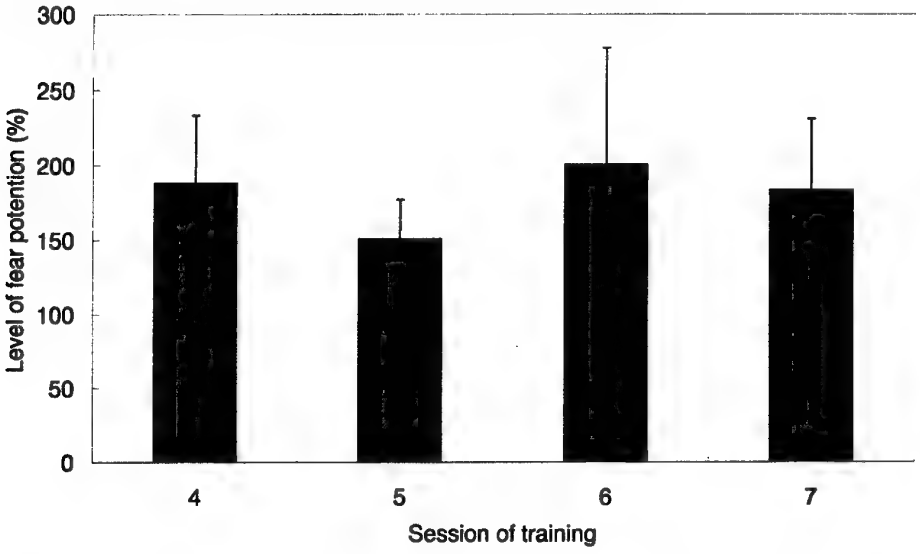


Figure 6: *Fear potentiation of the startle response determined at different points in time. Sessions 4 and 5 are successive weeks, thereafter the animals had a 1.5 week rest, then they were tested again (session 6) without preceding training. Thereafter the animals were trained again and tested the third day (session 7).*

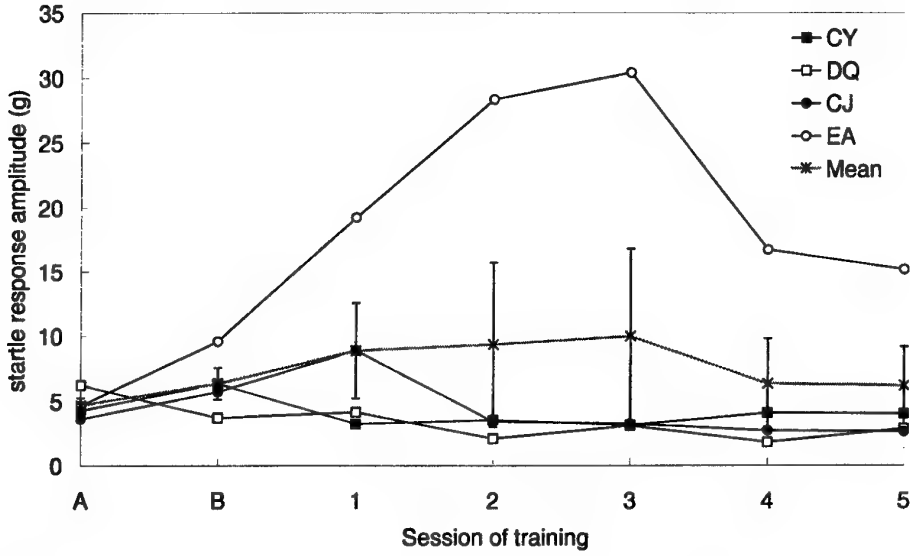


Figure 7: *Baseline startle responses of 4 individual animals (as indicated on the right of the figure) as well as the mean (± sem) of these responses over time. Session A and B are from the experiments determining respectively the optimal startle stimulus intensity and from the experiments on PPI. Sessions 1 to 7 represent the 5 control startle responses after the animals were subjected to the fear conditioning. At session 6, no conditioning was done. The baseline startle was more or less stable in 3 out of the 4 animals, but increased tremendously in the fourth animal (EA) after fear conditioning started. The session numbers are the same as indicated in Figure 6.*

3.1.4 Optimization of the fear potentiation protocol

The next series of experiments was aimed at improving of the training protocol used for the fear potentiation of the animals. Two different protocols were tested:

Protocol 1: two training sessions, 15 trials each, dark and light periods are interchanged, dark period is 4 seconds, during the last 0.5 second the aversive stimulus is given (350 μA, scrambled over the gridfloor). The light period had a duration of 1-2 minutes. The test session took place on the third day;

Protocol 2: in principle this method is similar to protocol 1, however, as a 'reminder' during the test session, 5 aversive stimuli were given randomly dispersed over time during a dark period.

Training and test sessions were always performed on the same day of the week. A total of 8 monkeys were used to test for differences between the two test protocols (4 for each group); however, over time a number of monkeys had to be used for other (lethal) experiments, therefore, the 'n' decreases over time. At the end of the experiment, n was 2 for protocol 1 and 3 for protocol 2. Apart from the last 3 sessions, in which the number of animals was too low to draw any definitive conclusions, it appeared that there were only slight differences between the two treat-

ment groups (Figure 8), although the mean FPS was always larger in the group trained with reminder trials. Therefore, we decided to use the 'conservative' approach in our experiments, i.e. without 'reminder' trials.
In these experiments, a mean level of fear potentiation of about 180-250% was found which is similar, as in the earlier experiments.

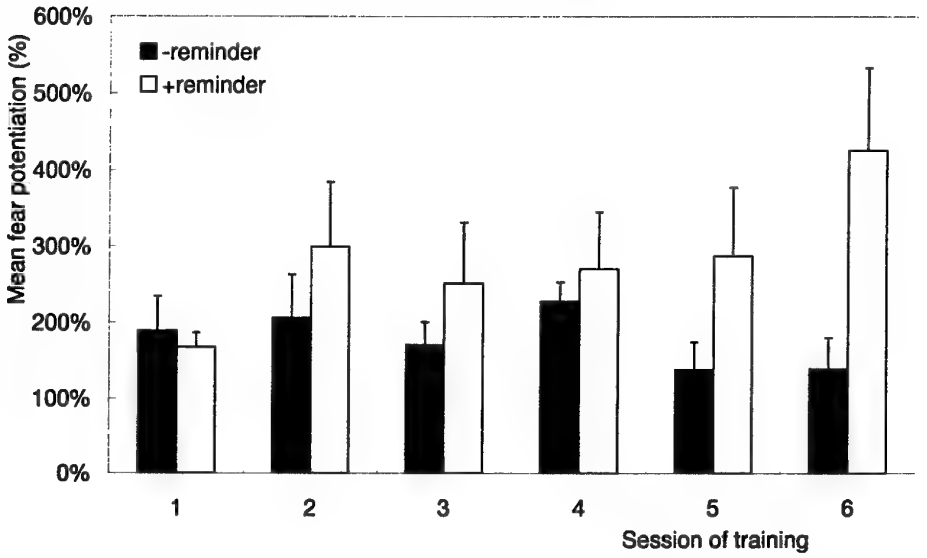


Figure 8: Mean startle responses with or without 'reminder' trials as indicated in the text. There were only slight differences between the two approaches.

3.1.5 Effect of background noise on PPI

The next experiment was aimed at determining the effect of a constant background noise (in this case 65 dB) on the PPI of the startle. Under the hitherto used recording conditions, i.e. without a constant noise, the level of the background noise varied between 40 and 55 dB. The startle reaction was determined at three levels of the pre-pulse (70, 75 and 80 dB) and with or without noise. In the first experiment, only signals with pre-pulse were recorded (due to a computer error), therefore, we had no control startle and hence no level of PPI. The results show no effect of the level of background noise when 75 or 80 dB pre-pulses were used, although with a pre-pulse of 70 dB, a much larger signal was found (Figure 9). At the moment, this effect cannot be explained. It is possible that a sound stimulus that is only 5 dB higher than the background noise is too weak to lead to any pre-pulse inhibition. If this is the case, the startle signal with the 70 dB pre-pulse should be (almost) equal to the control startle. In any case a constant background noise of 65 dBA was used in all the following experiments.

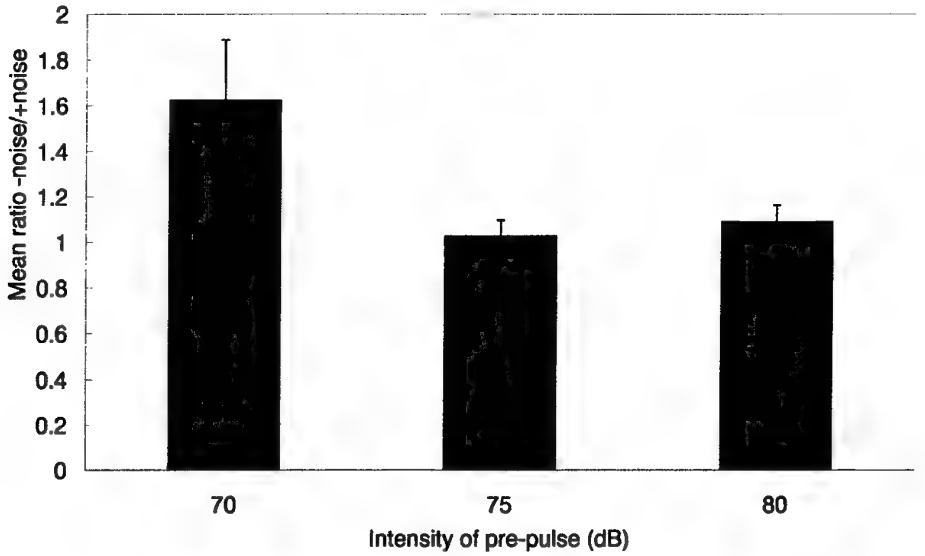


Figure 9: Lack of effect of a constant level of background noise on the level of PPI.

3.2 Drug effects

3.2.1 Effects of anxiolytics on the level of fear potentiation.

a Behavioural observations

No obvious effects were observed after saline injection. In the case of Haloperidol treatment, all animals showed a, dose-dependent, decrease of activity. A similar picture emerged after treatment with Buspiron. The effects of Diazepam were most pronounced: apart from the clear sedative effects of the drug, in 3 out of 4 animals diazepam induced, at the highest dose level, a clear unstable gait. Both sedation and unstable gait lasted for several hours. Fluvoxamine treated animals seemed to be somewhat slow; at the highest dose level, one of the two males had an erection.

b Stability of the startle response and fear potentiation over time

The protocol was chosen in such a way that at 5 points in time (see table below), the animals were given saline injections as a control for stability over time. The mean control and fear-potentiated startle responses are presented in Figure 10.

Table 1: Saline treatments.

Animal	Experimental session with saline treatment				
GT	1	4	8	11	16
GU	2	5	9	13	17
GV	1	5	8	12	16
GW	2	6	10	14	17

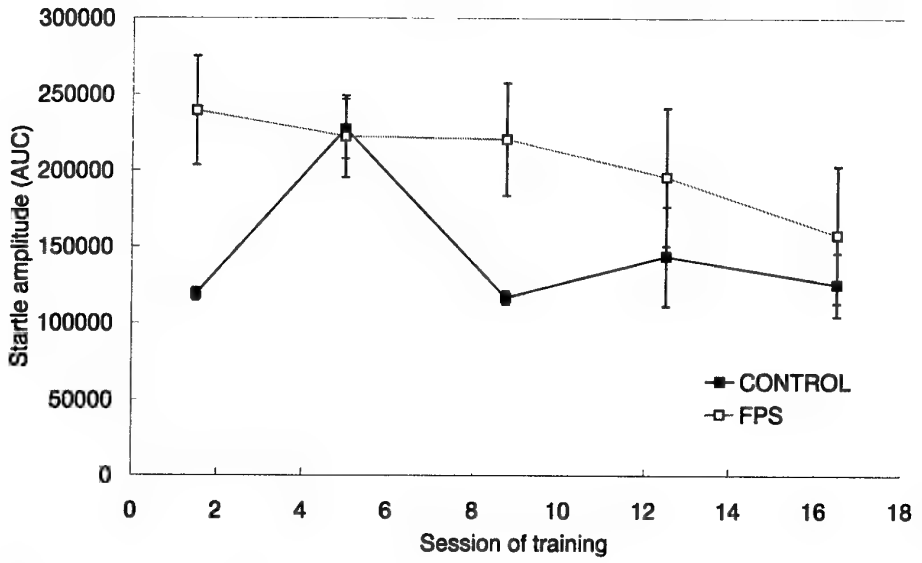


Figure 10: Effect of saline on baseline and fear-potentiated startle. In the training sessions around 5, the procedure was performed by another biotechnician. While leaving the FPS unaltered, it lead to an increase of the control startle to the level of the FPS.

A repeated measures Anova showed that the control startle appeared to be significantly larger in the second session as compared to the other 4 sessions ($F(3,19)=4.8$; $p<0.02$; $p<0.05$ Newman-Keuls post-hoc test). In this particular test session, the experiments were performed by another experimenter than usual. There were no significant differences between the levels of fear potentiation between the other test-sessions with saline. In view of this 'experimenter artefact', we decided to omit all data obtained in this particular period from the analysis.

c Effects on the control and FPS startle

The data were statistically tested using an ANOVA because of the missing values introduced by the artefact that had to be corrected for. Neither Diazepam nor Fluvoxamine had any effect on the startle amplitudes, neither in the control nor in the FPS situation. There was a clear effect of treatment both by Buspiron ($F(3,11)=7.3$ and 9.0; $p<0.01$ for the control startle and FPS, respectively) and Haloperidol ($F(3,9)=26.15$; and 5.05; $p<0.03$ for the control startle and the FPS, respectively). These data are shown in Figure 11.

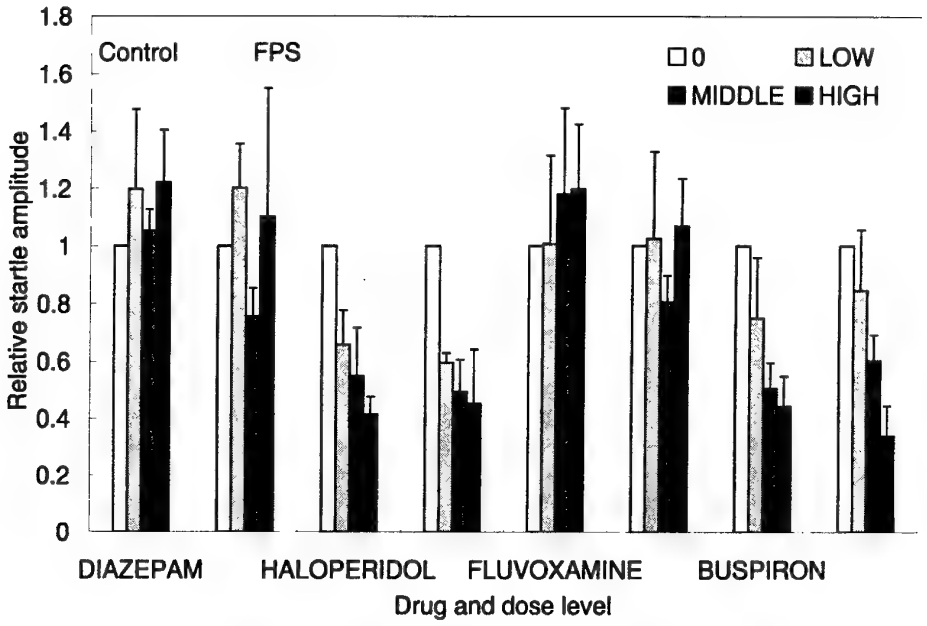


Figure 11: Effect of Diazepam, Buspiron, Haloperidol and Fluvoxamine on control (left) and FPS startle (right). In this and the following figure, the startles are given relative to the startle after injection of saline.

d Drug effects on FPS

First of all, it should be made clear that none of the mentioned effects on the fear potentiation of the startle were significant at the p=0.05 level (as tested with an ANOVA). Therefore, at best the data presented may give some indication about possible drug effects. In Figure 12, the effects of the different drugs on fear potentiation are presented. There appear to be three different effects: a) Haloperidol does not seem to have any effect on the fear-potentiated startle response. Since Haloperidol was used as the negative control in these experiments this was expected; b) Fluvoxamine seemed to have only a marginal effect; c) Diazepam does seem to have some (dose-response) effect, although the effect was not statistically significant; d) Buspiron showed a rather peculiar dose response curve: at lower dosages the fear potentiation seems to increase, whereas at higher dose levels, a slight reduction of the fear potentiation is found.

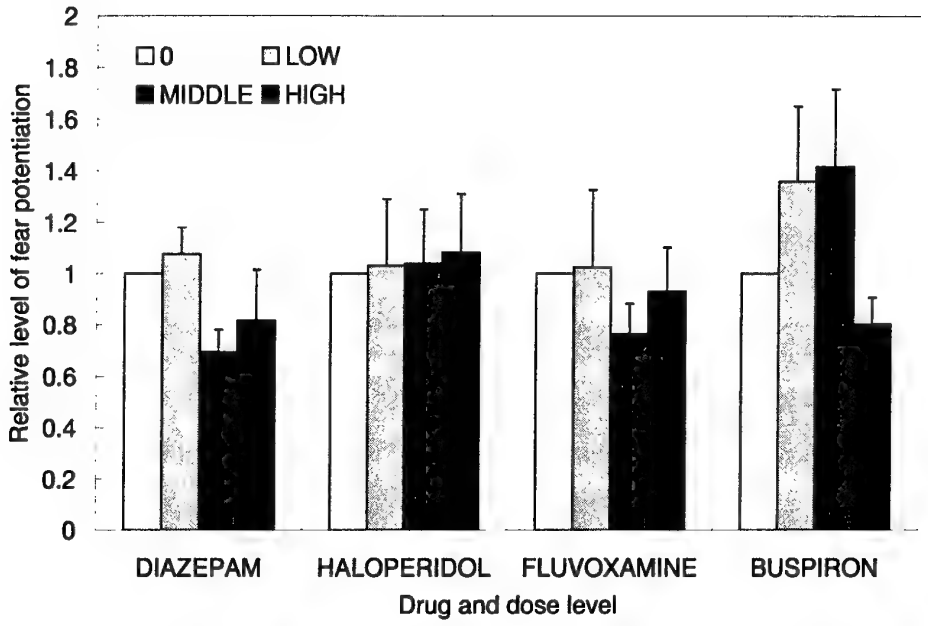


Figure 12: Effect of Diazepam, Buspiron, Haloperidol and Fluvoxamine on the level of fear potentiation.

3.2.2 Effects of hallucinogenics on PPI

a Behavioural observations

No clear symptoms were found after injection of Amphetamine, although some animals may have been somewhat more active at the highest dose. Behavioural effects were much more clear after injection of the other three drugs. After Quinpirol, animals were clearly more active. In addition, the animals showed an increased salivation and water intake. The effects of Ketamine and Diazepam were opposite to that of Quinpirol: after the highest dose levels the animals showed ataxia; in addition, some sedation occurred.

b Effects on the control startle amplitude

The control startle amplitudes are shown in Figure 13. In this case, no significant effects were found. Only Quinpirol ($p=0.07$, repeated measures Anova) showed a dose-dependent increase of the startle amplitude, the other three drugs showed no effect at all.

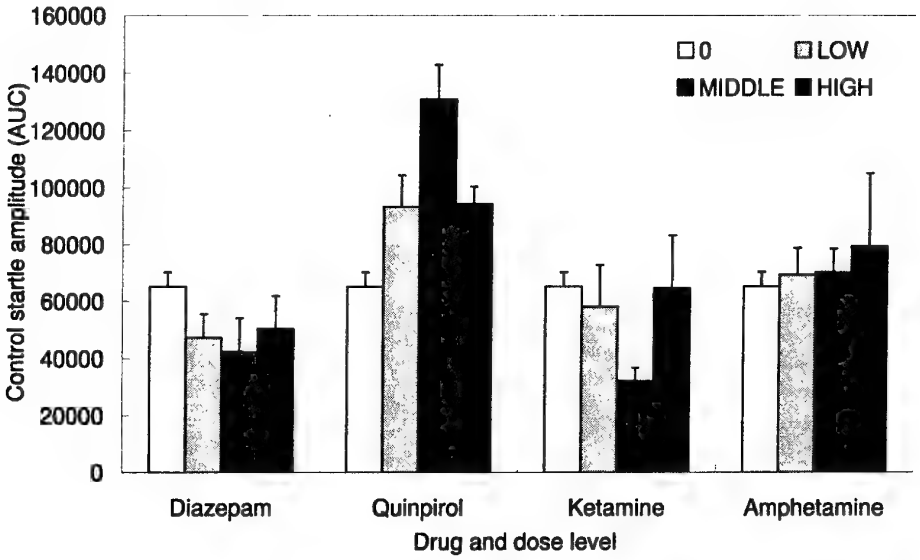


Figure 13: Effect of Quinpirol, Ketamine, Diazepam and Amphetamine on the baseline startle in the PPI experiments.

c Effects on PPI

The effects on the PPI elicited by a pre-pulse of 77 dB are shown in Figure 14. At the lower pre-pulse, no effect was visible. Again, none of the effects were significant on the $p<0.05$ level. However, there seemed to be a tendency for Ketamine ($p=0.07$) and may be Quinpirol to lead to a dose-dependent increase of the PPI.

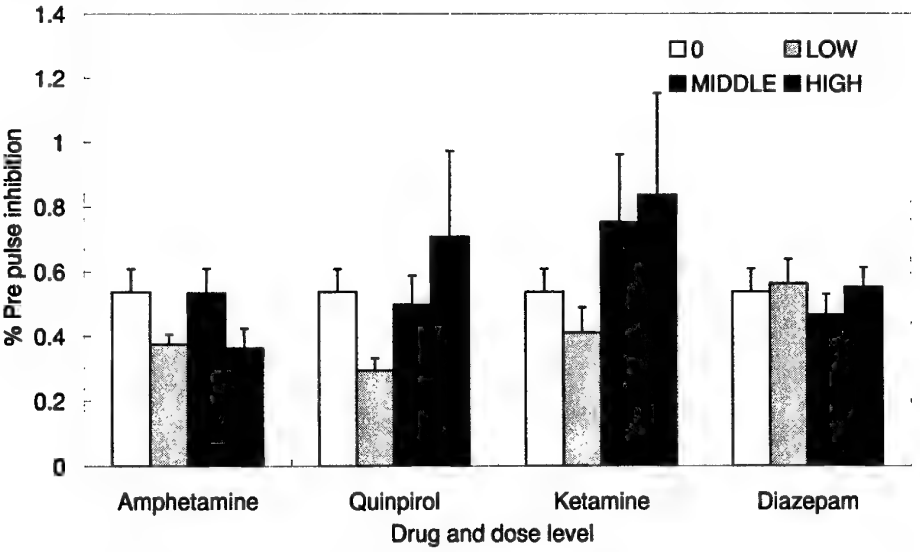


Figure 14: Effect of Quinpirol, Ketamine, Diazepam and Amphetamine on the level of PPI.

4 Discussion

In this study, we describe a set-up for the recording of auditory startle response in marmoset monkeys. To our best knowledge, no work of this kind has been performed yet in monkeys. Our first goal seems to have been reached: it is possible to measure the aucoustic startle response of marmoset monkeys in a reliable way. Not surprisingly, marmosets do show a similar pre-pulse inhibition of the acoustic startle reaction as described for many other species (Hoffman and Ison, 1980). This means that it should be possible to use this method, for instance, for the determination of the anti-psychotic activity of compounds.
In addition, marmosets also seem to show fear potentiation of the startle reflex. However, there is a rather large interindividual variation between different animals. For the purpose of drug testing, it might be necessary to select only those animals showing a large fear potentiation of the startle response. Both in the initial experiments and in the validation experiments, this problem occurred. Some animals apparently show only low levels of fear potentiation. The small size of the current marmoset population in our animal facilities prevented a choice from a large group of animals. Evidently, it would have been better if we could have selected those animals with the largest levels of FPS from a large group of marmosets. Unfortunately, this was not possible. An additional potential problem may be that the fear potentiation procedure in itself leads to such a level of anxiety in the animals that the control startle (i.e. the startle in 'the light' on situation) is already enhanced. This would reduce the level of fear potentiation that can be reached. Alternatively, the conditioning of an animal might lead to a general fear of the startle set-up: this opposed to a fear of only the 'light off' situation. We did find evidence of this phenomenon: the control startle response of one of the animals (EA) increased tremendously (Figure 7). This might have affected the level of fear potentiation in this animal. The animal did not show fear potentiation in sessions 1 and 2, however, when its control startle decreased again (sessions 4 and 5), the animal showed a clear fear potentiation of the startle response. Obviously, the animal had not adjusted to the procedure during the first three startles. The way in which the procedure is performed also seems to be of importance. From Figure 10, it appears that the animals were stressed when they were handled by another biotechnician than usual. The level of the control startle around training session 5 was similar to that of the FPS.

The effects of several anxiolytics on fear potentiation were tested. All these drugs were given at doses that were effective: first, both Buspiron and Haloperidol affected the control and FPS; secondly, all drugs had clear behavioural effects. Leaving aside that none of the effects on the FPS were significant, the following trends are visible. As to be expected, Diazepam seems to reduce the fear potentiated startle, whereas the anti-depressant Fluvoxamine had only a marginal, if any, effect. Buspiron seems to have a biphasic dose-response curve: it enhanced the FPS at low dose levels, whereas at the highest dose, some reduction of fear potentiation

might be seen. In rats, usually a clear reduction of fear potentiation is seen (e.g. Nevins and Anthony, 1994) without an effect on the baseline startle. In addition, Buspiron also seems to be effective in the human threat test with marmosets (Barnes et al., 1991; Costall et al., 1992). Interestingly, the activity of Buspiron might parallel the activity of this drug in humans. In humans, usually it is necessary to take the drug for a period of at least two weeks before it starts to take effect. In the period before the drug becomes effective, pathological fear may be enhanced. The anti-psychotic Haloperidol had no effect on the FPS.

The effects of Diazepam, Amphetamine, Quinpirol and Ketamine were tested on the PPI. Diazepam seemed to have no effect on the startle amplitude, neither on the amplitude of the control nor on the amplitude of the PPI startle. However, it clearly affected behaviour.
Only few studies have reported on the effects of Amphetamine in monkeys. These studies suggest that in our case, Amphetamine may have been applied in a dose that is too low to exert an effect: we found no clear behavioural abnormalities in the animals even at a dose of 1 mg/kg. In addition, D' Mello et al. (1985) found no effect of d-Amphetamine in their test on hand-eye coordination up to a dose level of 4 mg/kg. Although this test is clearly different from our test system, it might indicate that the dose level of Amphetamine was indeed too low to expect an effect.
Quinpirol and Ketamine seemed to have some effect: Quinpirol enlarged the control startle amplitude and the PPI slightly; Ketamine had no effect on the control startle but it did seem to enhance the PPI.

In conclusion: although we cannot be completely satisfied with the results of the present study, the general trend seems to be that the drugs that should have an effect (be it anxiolytic or hallucinogenetic) show at least the trend to do so. The interindividual variation is a point of concern, however, this seems also the case in rats at least with respect to the fear potentiation. One simple way to decrease this variability seems a selection of animals with the largest FPS. In addition, the number of animals that are used in the test could be increased. Alternatively, an improvement of the procedure to induce FPS in the animals might be successful. For instance, the total number of training trials could be enlarged in some animals to give the desired effect, or in some animals 'reminder trials' may be given during the test.

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6 Authentication

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Set-up of a system to reliably measure the startle response in marmoset monkeys; application in animal models of anxiety and psychosis

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15. ABSTRACT (MAXIMUM 200 WORDS (1044 BYTE))

The acoustic startle response is a reflex motor response elicited by a sudden loud sound. In two psychiatric disorders, the startle reflex is altered. Normally, the startle response is decreased appreciably when a low intensity sound stimulus is given shortly before the startle eliciting stimulus. This prepulse inhibition of the startle is nearly absent in patients suffering from schizophrenia. In addition, the startle response is increased during periods of anxiety. In this study, a system is described by which the acoustic startle response in marmoset monkeys may be recorded in a reliable way. In using this system, it could be shown that marmosets possess a pre-pulse inhibition (PPI) of the ASR, similar as in other species. Furthermore, it was shown that a fear potentiation of the startle response may be elicited in the marmoset. Potentially, this system may be used for testing anti-psychotic or anxiolytic activity of drugs in primates. In this study, a series of drugs with a known activity on PPI or fear potentiation of the ASR was tested to validate the system. The effects of Buspiron and Diazepam were tested as examples of drugs with a known anxiolytic effect. In addition, Fluvoxamine and Haloperidol were used in these experiments. Amphetamine, Ketamine and Haloperidol were used as drugs to affect the PPI; in these experiments Diazepam was used as a negative control. These experiments were performed in a randomized, cross-over design. In the fear potentiated startle experiments, four animals were used. They received, following a training period to establish a stable baseline fear potentiation, three dose levels of each of the drugs. In addition, the animals were injected with saline 5 times spread evenly over the total duration of the experiment to test the stability of the baseline fear potentiation. The four animals used in the PPI group were subjected to a similar dosing schedule. Buspiron, Haloperidol and possibly Quinpirol had an effect on the control startle reponse. Although none of the effects of the drugs on either FPS, or PPI were statistically significant, Diazepam and to a lesser degree Fluvoxamine tended to decrease the FPS. Buspiron seemed to have a biphasic effect on the FPS whereas Haloperidol had no effect. Ketamine and Quinpirol seemed to reduce the PPI and Diazepam had no effect. Amphetamine probably was given at too low a dose to affect PPI.

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